ASBMT
American Society for Blood and Marrow Transplantation

Peripheral Blood CD34$^+$ Cell Enumeration as a Predictor of Apheresis Yield: An Analysis of More Than 1,000 Collections

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The role of the peripheral blood (PB) CD34$^+$ cell count in predicting the CD34$^+$ cell yield in hematopoietic progenitor cell apheresis collections is well established. However, sometimes unexpectedly poor CD34$^+$ cell yields are obtained. To determine the effect, if any, of a range of factors on the ability of the PB CD34$^+$ count to predict collection CD34$^+$ cell count, we performed a retrospective analysis on consecutive hematopoietic progenitor cell apheresis collections between 2004 and 2008. Factors investigated included mobilization regimen, PB white blood cell count, body weight, and disease. After exclusion of collections involving apheresis complications, a total of 1,225 PB CD34$^+$ cell results with corresponding collection CD34$^+$ cell results from 458 patients were analyzed. Although differences in the median PB CD34$^+$ cell counts and collection CD34$^+$ cell counts were seen between mobilized collections with chemotherapy plus granulocyte colony-stimulating factor and those with granulocyte colony-stimulating factor alone, the predictive capability of the PB CD34$^+$ cell count for the collection CD34$^+$ cell yield remained similar. Although poorer collection efficiencies were observed in the myelodysplastic syndrome/myeloproliferative disorder diagnostic subgroup, our findings confirm that PB CD34$^+$ cell analysis remains a powerful and irreplaceable tool for predicting hematopoietic progenitor cell apheresis CD34$^+$ cell yield.

Biol Blood Marrow Transplant 18: 763-772 (2012)

KEY WORDS: CD34, HPC-A collection, Mobilization, WCC, Weight, Disease

INTRODUCTION

High-dose chemotherapy and autologous hematopoietic progenitor cell (HPC) transplantation is established therapy for various hematologic cancers and solid tumors [1-6]. With the availability of growth factor-based mobilization regimens, peripheral blood (PB) collections by apheresis (HPC-A) have generally replaced bone marrow as the preferred source of HPCs [7]. HPC-A collection has proven to be more convenient, safer, and more cost-effective while demonstrating more rapid hematopoietic recovery with lower microbial contamination rates [6-10]. However, HPC-A collection and cryopreservation remains a resource-consumptive procedure. Cost analyses vary, but the reported cost range in one study was US $9,987-$26,263 per patient for the evaluation, mobilization, harvesting, and cryopreservation phases alone [11]. The wide variation in cost is explained in part by the variability in number of collections per patient. Thus, reliable prediction of the HPC-A is a valuable tool to minimize costs by avoiding unnecessary collections.

Detection of the CD34 antigen on the surface of leukocytes has proven to be a reliable surrogate marker for identification of self-renewing, pluripotent HPCs and has generally replaced the cumbersome colony-forming unit assay for granulocytes and macrophages [12,13]. Assessment of apheresis products by measuring CD34$^+$ cell counts by flow cytometry evaluates the most primitive HPCs along with maturing, lineage-committed progenitors. Doses $>2 \times 10^6$ CD34$^+$ HPCs/kg of patient body weight have been found to be predictive of both short-term and long-term hematopoietic recovery after high-dose chemotherapy [14,15].

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Financial disclosure: See Acknowledgments on page 772.
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Received August 10, 2011; accepted October 1, 2011

1083-8791/$36.00
doi:10.1016/j.bbmt.2011.10.002

The role of PB $CD34^+$ cell counts in predicting the optimal timing of HPC-A collection is well established, as is its contribution to achieving an adequate $CD34^+$ dose [16-23]. Quantitation of $CD34^+$ HPCs is generally available within 1 hour after collection. Moreover, the single-platform assay demonstrates low interlaboratory and intralaboratory variability [24]. In general, centers that use PB $CD34^+$ cells as a trigger for HPC-A collection do not start apheresis until a minimum PB $CD34^+$ cell threshold is reached. At our institution, we previously showed that a PB $CD34^+$ count of 5.0×10^6/L reliably predicts an HPC-A product $CD34^+$ cell count of $>0.5 \times 10^6$/kg [22]. Thus, our minimum $CD34^+$ cell infusion dose of 2.0×10^6/kg should be obtained within a maximum of four HPC-A collections. Despite this, PB $CD34^+$ count as a predictor of product yield can be unreliable, and sometimes unexpectedly poor $CD34^+$ cell yields are obtained, referred to as *poor $CD34^+$ collection efficiency* (CE). A range of factors are known to contribute to CE, including optimal apheresis performance (instrument- and operator-associated), patient body weight, PB WBC count (WCC), hematocrit value, serum albumin level, diseases, and mobilization regimen [25]. Although some of these factors are known to have an effect on mobilization efficiency, their effect on CE is not well understood.

To evaluate the effect, if any, of a range of factors on the ability of the PB $CD34^+$ count ($\times 10^6$/L) to predict the collection $CD34^+$ cell yield ($\times 10^6$/kg), we performed a retrospective analysis of consecutive HPC-A collections during the period from 2004 to 2008. Our primary aim was to determine how closely the PB $CD34^+$ cell count predicted the HPC-A $CD34^+$ cell yield across a range of clinical and biological variables. We sought to confirm our previous work (and other published data) indicating that PB $CD34^+$ count reliably predicts HPC-A $CD34^+$ cell yield, as well as to stratify our data into a number of clinical variables to assess whether these variables have a negative affect on this predictive ability.

METHODS

Patients and Assessed Variables

A total of 1,297 HPC-A collections obtained from 458 patients during the 5-year analysis period were included in the initial analysis. Collections that incurred unexpected complications during apheresis, resulting in reduced processing volume, were excluded from analysis (n = 72). The median patient age was 55 years (range, 17-81 years). Other patient characteristics are summarized in Table 1.

Mobilization

The mobilization regimen for 305 patients (810 individual HPC-A collections) involved a range of chemotherapy agents plus granulocyte-colony stimulating factor (G-CSF [filgrastim]; Amgen Australia, XXX), the regimen for 153 patients (415 individual HPC-A collections) involved filgrastim alone. The filgrastim was administered as daily s.c. injections at a dose of 10 μg/kg. For filgrastim-alone mobilizations, filgrastim was started on day 1, and PB $CD34^+$ cell monitoring was initiated on day 4. After the chemotherapy + filgrastim mobilization, filgrastim was administered at 10 μg/kg/day starting the day after the last day of chemotherapy. PB $CD34^+$ counts were monitored according to expected leukocyte recovery after myelosuppressive chemotherapy, and HPC-A collection was initiated once PB $CD34^+$ counts were $\geq 5 \times 10^9$/L, in accordance with our unit's policy.

Table 1. Patient Characteristics (n = 458)

Characteristic	Patients, n (%)
Sex	
Male	284 (62.0)
Female	174 (38.0)
Diagnosis	
NHL	193 (42.1)
Plasma cell dyscrasias	156 (34.1)
Acute myelogenous leukemia	25 (5.5)
Hodgkin lymphoma	20 (4.4)
Myeloproliferative neoplasms	13 (2.6)
Sarcoma	11 (2.4)
Solid tumor	10 (2.2)
Chronic lymphocytic leukemia	9 (2.0)
Acute lymphoblastic leukemia	8 (2.7)
MDS	3 (0.7)
Other	10 (2.2)

Apheresis Collection

Autologous HPC-A collections were performed by the apheresis unit under current good manufacturing practices (cGMP) conditions (Australian Therapeutic Goods License 149827) using Cobe Spectra (Cobe, Denver, CO) and Haemonetics MCS+ (Baxter Healthcare, Deerfield, IL) apheresis machines. Collection was performed until 2 blood volumes were processed with the Spectra device (continuous processing) or 1.5 blood volumes were processed with the MCS+ device (discontinuous processing).

Collection episode characteristics are summarized in Table 2. Factors analyzed for potential impact on prediction were PB WCC (<20, 20-50, and $>50 \times 10^9$/L), mobilization regimen (filgrastim alone or filgrastim + chemotherapy), patient body weight (<70, 70-100, and >100 kg), and diagnosis (non-Hodgkin lymphoma, plasma cell dyscrasia, or other).

HPC-A Cryopreservation

Autologous HPC-As were processed under cGMP conditions within 24 hours of collection. Volume reduction of the initial apheresis product was performed by centrifugation and removal of plasma and platelets. The platelet-poor autologous plasma generated as a byproduct of this process was used whenever possible

Table 2. Collection Frequencies (n = 1,225)

Variable	Mean	Median	Range	Category	Collections, n (%)
PB WCC $\times 10^9$/L	29.1	26.6	0.9-202	<20	453 (37.0)
				20-50	608 (49.6)
				>50	164 (13.4)
Mobilization				Filgrastim alone	415 (33.9)
				Filgrastim + chemotherapy	810 (66.1)
Weight, kg	78.1	78	43-136	<70	358 (29.2)
				70-100	780 (63.7)
				>100	87 (7.1)
PB CD34+ cells, $\times 10^6$/L	61.2	21.2	0.2-1980.7	Low: <20	593 (48.4)
				Moderate: 20-50	281 (22.9)
				High: >50	351 (28.7)
Collection CD34+ cells, $\times 10^6$/kg	4.04	1.48	0.01-117.8		

to provide the protein content of the cryoprotectant. When insufficient autologous plasma was available, Albumex 20 (CSL, Melbourne, Australia) was used. The cell concentration was adjusted to result in a final product with a total WBC concentration of $<200 \times 10^6$/mL. A viable CD34+ cell count was performed on this volume-reduced buffy coat, with the result reported as $\times 10^6$/kg patient body weight. The volume-reduced buffy coat was suspended in 10% DMSO solution with approximately 2% protein and then frozen at a controlled rate and stored in vapor-phase nitrogen above liquid nitrogen until required for use.

Flow Cytometric CD34+ HPC Determination

Viable PB CD34+ cell collection CD34+ cell counts were performed using a single-platform method based on the ISHAGE gating strategy [26-29] (Figure 1) using a FACSCalibur flow cytometer (BD, San Jose, CA). In brief, a volume of whole blood or buffy coat was incubated with 10 μL of CD45 FITC (clone J33; Immunotech, Marseille, France), 10 μL of CD34 PE (Immunotech clone 581), and 10 uL of 7AAD (Immunotech) for 15 minutes at room temperature in the dark. Red cells were lysed with ammonium chloride (Immunotech) for 10 minutes. An equal volume of well-mixed FlowCount fluorospheres (Beckman Coulter, Fullerton, CA) of known concentration was added, and data were acquired on the flow cytometer without washing. PB CD34+ and collection CD34+ cell counts were calculated and reported as cells $\times 10^6$/L and cells $\times 10^6$/kg body weight, respectively.

Statistical Analysis

Patient demographic data were summarized using descriptive statistics, including the mean, median, and range for data measured on a continuous scale and counts and percentages for categorical data. Mobilization was classified into two mobilization regimen groups (filgrastim alone vs filgrastim + chemotherapy). Weight was classified into three groups (<70 kg, 70-100 kg, and >100 kg), as were WCC count ($<20 \times 10^9$/L, 20-50 $\times 10^9$/L, and $>50 \times 10^9$/L), PB CD34+ cell count ($<20 \times 10^6$/L, 20-50 $\times 10^6$/L, and $>50 \times 10^6$/L), and diagnosis (non-Hodgkin lymphoma [NHL], plasma cell dyscrasias [principally multiple myeloma (MM)], and other). Both PB CD34+ cell and collection CD34+ cell counts showed a skewed (nonnormal) distribution and thus were transformed to the natural logarithmic scale before any statistical models were fitted. Univariate linear regression to test for associations between PB CD34+ cells and collection CD34+ cells was performed separately for each category of the following factors: mobilization regimen, weight category, WCC category, and diagnosis. The goodness of fit was assessed using R^2. Regression models were compared using the χ^2 goodness-of-fit statistic.

Multivariate linear regression was performed on the aforementioned variables. The final model was obtained considering a stepwise procedure in which the inclusion of a variable was assessed by the likelihood ratio test of the nested models. The residuals were analyzed to evaluate the adequacy of the final model.

In addition, CE was calculated using the following formula:

$$\text{Collection efficiency (\%)} = \frac{\text{Total CD34+ cells obtained from apheresis}}{\text{PB CD34+(ul)X apheresis process volume(Lt)}}$$

Collections with a PB CD34+ cell count $>10 \times 10^6$/L and a CE <20% (arbitrarily set lower limit) were tabulated according to the foregoing characteristics and analyzed for possible trends that might contribute to low CE. On these, boxplots were drawn to demonstrate trends where relevant, with the two-tailed Student *t*-test used to determine significance.

RESULTS

After elimination of 72 collections involving unexpected cessation or prolongation of apheresis, 1,225 PB CD34+ cell results and the corresponding collection

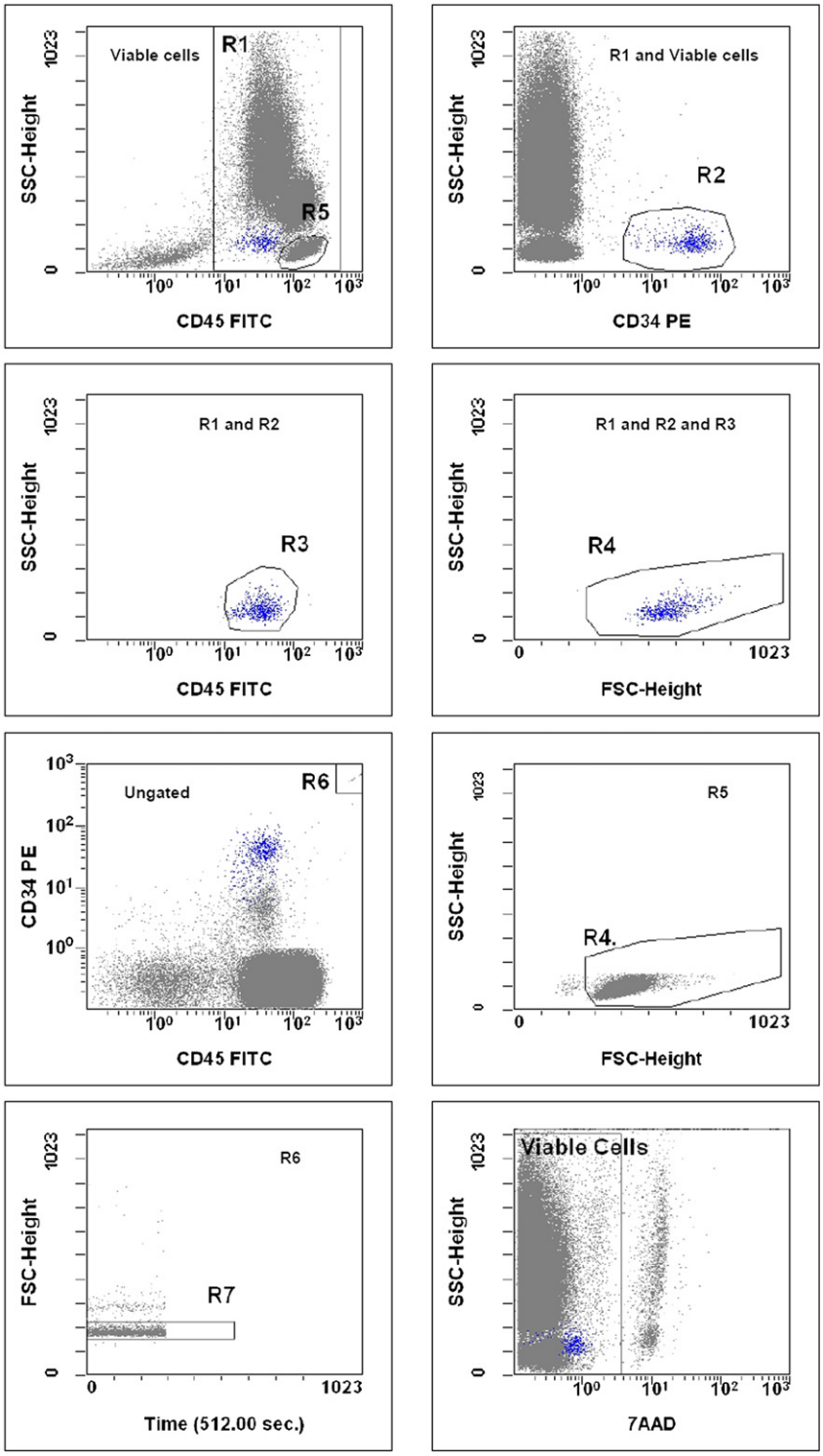

Figure 1. Single-platform CD34 ISHAGE-based gating strategy. Viable cells were analyzed using a gate based on 7AAD exclusion. A gate was then drawn on CD45$^+$ cells (R1) to exclude noncellular debris and unlysed red cells. A gate was then drawn to capture a discrete cluster of CD34$^+$ cells with low side scatter (R2). These cells were further refined using a homogenous cluster based on intermediate CD45$^+$ cell expression (R3), and the CD34$^+$ HPCs (R4) were finally defined using intermediate forward scatter (final CD34$^+$ HPCs compared with lymphoid population follicular stem cells). The number of events in this R4 gate were compared with the number of events counted in the singlet bead gate (R7) to derive the reported CD34$^+$ cell count.

CD34$^+$ cell results (from 458 patients) were analyzed. Summary statistics for the variables analyzed in these 1,225 samples are presented in Tables 2 through 4. Nine patients with a PB CD34$^+$ cell count >10 $\times$ 10^6/L and CE <20% were identified; data for these patients are presented in Table 5.

Table 3. PB and Collection CD34+ Cell Results According to Collection Variables (n = 1,225)

Variable	n	PB CD34+ Cells, × 10^6/L			Collection CD34+ Cells, × 10^6/kg		
		Mean	Median	Range	Mean	Median	Range
Weight, kg							
<70	358	56.0	19.0	0.8-859.5	3.87	1.40	0.04-52.60
70-100	780	60.9	21.1	0.2-1980.7	3.98	1.46	0.01-117.80
>100	87	66.2	24.8	1.4-504.1	3.93	1.73	0.10-35.40
Mobilization							
Chemotherapy + filgrastim	810	75.9	29.2	0.2-1980.7	5.10	2.06	0.01-117.80
Filgrastim alone	415	27.1	10.0	0.3-526.4	1.59	0.78	0.01-28.64
Pre-WCC							
<20 × 10^9/L	453	62.9	21.9	0.2-1980.7	4.78	1.92	0.01-97.13
20-50 × 10^9/L	608	54.9	18.7	0.5-1608.0	3.39	1.20	0.03-117.80
>50 × 10^9/L	164	68.4	30.2	1.1-859.5	3.64	1.55	0.04-42.80
NHL	455	54.0	19.0	0.3-1980.7	3.77	1.31	0.03-97.13
Plasma cell dyscrasias	417	72.4	27.6	0.2-678.8	4.70	1.87	0.01-46.50
Other	353	57.1	19.7	0.9-1608.0	3.62	1.31	0.04-117.8

Univariate Linear Regression

Overall

The relationship between PB CD34+ cells and collection CD34+ cell yield is illustrated in Figure 2A and B. For all of the data analyzed, a significant linear relationship was shown between log collection CD34+ cell count and log PB CD34+ cell count. The fitted relationship between log collection CD34+ cell count and log PB CD34+ cell count is shown in Figure 2C, showing that a doubling of fluorosphere PB CD34+ cell count would lead to a $2^{0.9593}$ = 1.94-fold higher collection CD34+ cell count.

Log collection CD34+ and log PB CD34+ cell counts and effect of mobilization

Compared with the overall model, the effect of mobilization group (filgrastim alone vs chemotherapy + filgrastim) does not alter the fit. Although filgrastim + chemotherapy mobilization was associated with higher PB CD34+ cell counts (30.4 × 10^6/L vs 10.4 × 10^6/L) and collection CD34+ cell counts (2.1 × 10^6/kg vs 0.8 × 10^6/kg) compared with filgrastim-alone mobilization, the actual slopes are similar. The fitted regressions are shown in Figure 3.

Log collection CD34+ and log PB CD34+ cell counts and the effect of weight

The effect of patient body weight was analyzed by dividing the patients into three weight categories. Although small differences in the mean log PB CD34+ cell and log collection CD34+ cell counts among the three weight categories was shown, the rate of increase of did not differ significantly (ie, similar slopes, or parallel lines). The fitted regressions between log collection CD34+ cell and log PB CD34+ cell counts according to weight categories (<70 kg vs 70-100 kg vs >100 kg) are shown in Figure 4.

Log collection CD34+ and log PB CD34+ cell counts and the effect of WCC

The effect of PB WCC was analyzed by dividing patient data into three categories of WCCs. Again, although small differences in the mean log PB CD34+ cell and log collection CD34+ cell counts were seen among the three categories, the rate of increase of log collection CD34+ cells with log PB CD34+ cells did not differ significantly among the three WCC categories (ie, similar slopes or parallel lines). The fitted regressions between log collection CD34+ cells and log PB CD34+ cells according to WCC category (<20 × 10^9/L vs 20-50 × 10^9/L vs >50 × 10^9/L) are shown in Figure 5.

Log collection CD34+ cell and log PB CD34+ cell counts and the effect of diagnosis

The effect of diagnosis was analyzed by dividing patients into three categories: NHL (n = 455), MM (n = 417), and other (n = 353). Again, although a difference in the mean and median levels of log PB CD34+

Table 4. PB and Collection CD34+ Cell Results According to Mobilization Regimen: Effect of WCC

Variable	n	PB CD34+ Cells, × 10^6/L			Collection CD34+ Cells, × 10^6/kg		
		Mean	Median	Range	Mean	Median	Range
Chemotherapy + filgrastim mobilization	810						
Pre-WCC <20 × 10^9/L	385	70.4	28.1	0.2-1980.7	5.48	2.34	0.01-97.13
20-50 × 10^9/L	327	84.3	29.1	0.8-1608.0	5.15	1.87	0.05-117.80
>50 × 10^9/L	98	88.6	35.5	1.7-859.5	4.96	1.98	0.04-42.79
Filgrastim alone mobilization	415						
Pre-WCC <20 × 10^9/L	68	33.0	6.7	0.3-338.2	1.75	0.52	0.01-18.01
20-50 × 10^9/L	281	22.2	11.0	0.5-223.0	1.45	0.82	0.03-18.18
>50 × 10^9/L	66	46.5	17.8	1.3-526.4	2.24	1.07	0.05-28.64

Table 5. Collection Episodes With CE <20% and PB CD34$^+$ >10 $\times$ 10^6/L (n = 9)

Patient	Day of Collection	Diagnosis	Mobilization	Patient Weight, kg	Peripheral WCC, 10^9/L	PB CD34$^+$, 10^6/L	Collection CD34$^+$, 10^6/kg	Collection Efficiency, %
1	First of 4	MPD	Filgrastim alone	92	10.1	247.0	2.21	6.7
2	First of 3	MM	Chemotherapy + filgrastim	69	3.9	67.5	1.02	10.4
3	Second of 2	MM	Chemotherapy + filgrastim	53	24.6	561.9	9.77	13.2
4	Second of 2	MDS	Filgrastim alone	96	56.0	340.4	5.90	13.7
5	Third of 3	MPD	Filgrastim alone	91	69.8	126.1	2.22	13.9
6	First of 4	MM	Chemotherapy + filgrastim	73	34.6	39.5	0.76	14.5
7	Second of 2	MPD	Filgrastim alone	100	49.1	219.7	4.26	15.4
8	First of 4	NHL	Chemotherapy + filgrastim	82	49.5	38.7	0.94	17.8
9	Second of 3	NHL	Chemotherapy + filgrastim	67	53.5	159.5	5.10	19.9

cells and log collection CD34$^+$ cells among the three categories was demonstrated, the rate of increase of log collection CD34$^+$ cells with log PB CD34$^+$ cells did not differ significantly among the three diagnoses (ie, similar slopes or parallel lines). The fitted regressions between log collection CD34$^+$ cells and log PB CD34$^+$ cells according to diagnosis are shown in Figure 6.

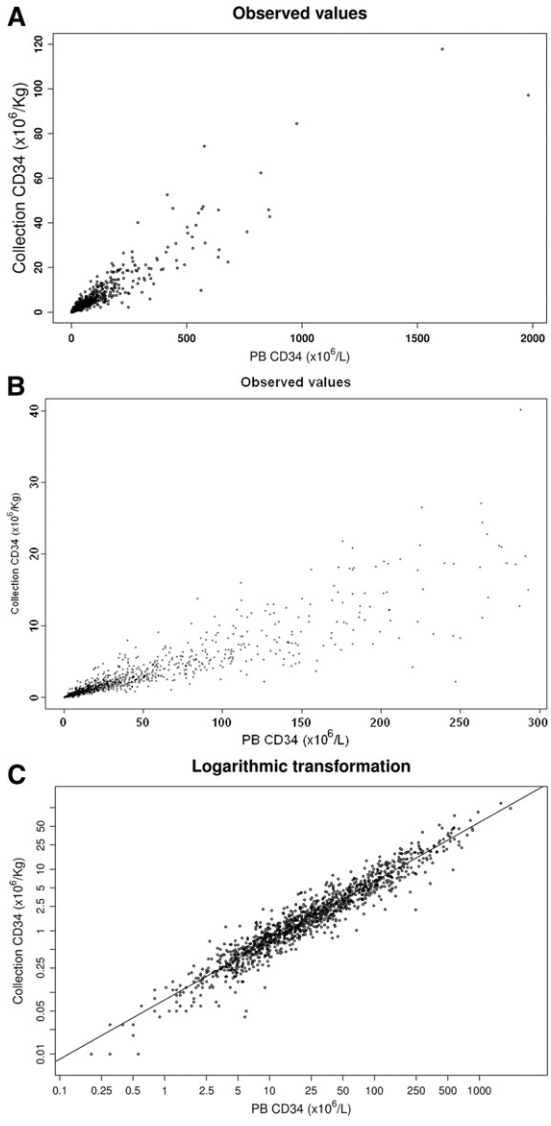

Figure 2. (A) Non–log-transformed PB CD34$^+$ cell counts versus collection CD34$^+$ cell counts. (B) Non–log-transformed PB CD34$^+$ cell counts versus collection CD34$^+$ cell counts for all samples with PB CD34$^+$ cell count <300 $\times$ 10^6/L. (C) Relationship between collection CD34$^+$ cell count and PB CD34$^+$ cell count for all samples (after logarithmic transformation); $y = -2.5712 + 0.9593x$; $R^2 = 91.9\%$; $P < .0001$.

Multivariate Modeling Considering Possible Confounders

The relationship between PB CD34$^+$ cell and collection CD34$^+$ cell counts was further investigated considering WCC, weight, mobilization, and diagnosis as potential confounders. Multivariate linear regression using a stepwise procedure was performed based on the preceding simple linear regression (Figure 2C). Mobilization and diagnosis did not

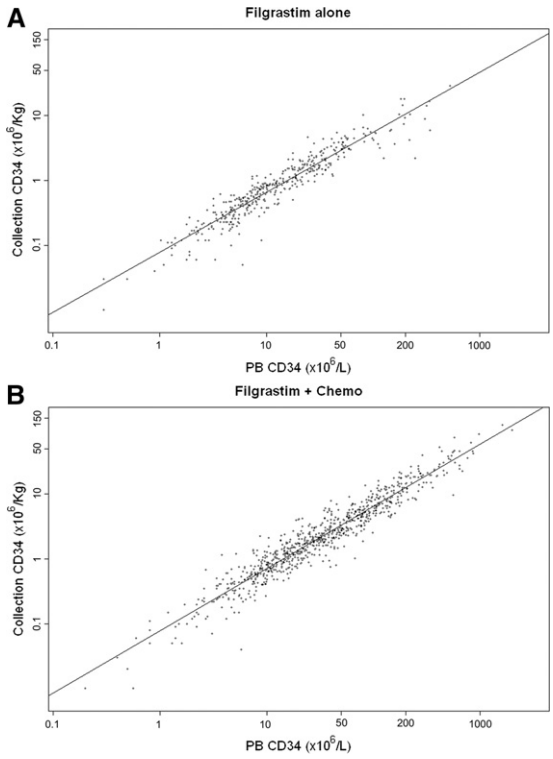

Figure 3. (A) Relationship between collection CD34$^+$ cell count and PB CD34$^+$ cell count for all samples from patients mobilized using filgrastim alone (after logarithmic transformation); $y = -2.5362 + 0.9234x$; $R^2 = 89.6\%$; $P < .0001$. (B) Relationship between collection CD34$^+$ cell count and PB CD34$^+$ cell count for all samples from patients mobilized using filgrastim + chemotherapy (after logarithmic transformation); $y = -2.5451 + 0.9597x$; $R^2 = 91.6\%$; $P < .0001$.

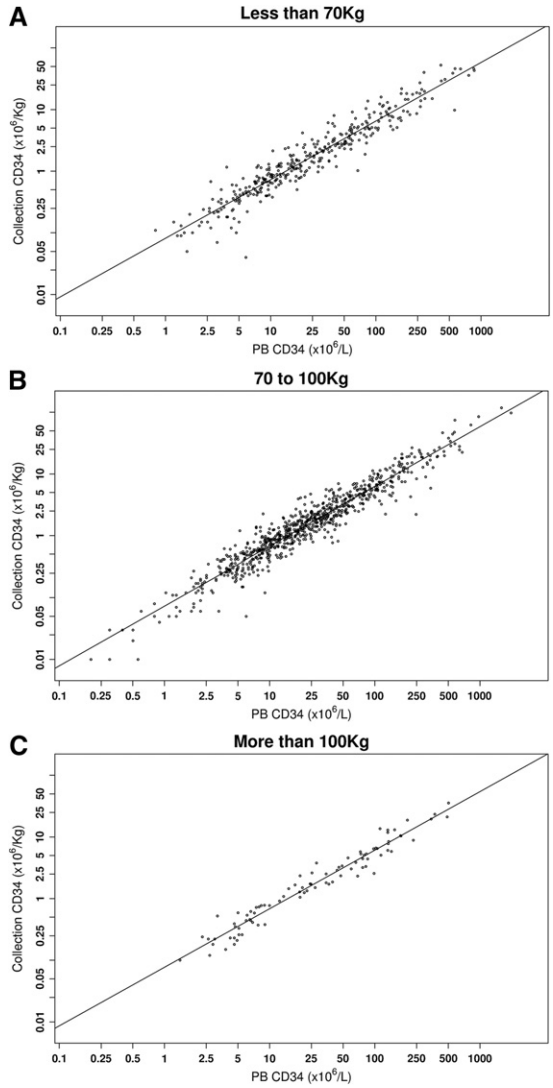

Figure 4. (A) Relationship between collection $CD34^+$ cell count and PB $CD34^+$ cell count for all samples from patients weighing <70 kg (after logarithmic transformation; $y = -2.5059 + 0.9508x$; $R^2 = 90.8\%$; $P < .0001$). (B) Relationship between collection $CD34^+$ cell count and PB $CD34^+$ cell count for all samples from patients weighing 70-100 kg (after logarithmic transformation; $y = -2.608 + 0.9651x$; $R^2 = 92.1\%$; $P < .0001$). (C) Relationship between collection $CD34^+$ cell count and PB $CD34^+$ cell count for all samples from patients weighing >100 kg (after logarithmic transformation; $y = -2.5518 + 0.9466x$; $R^2 = 94.9\%$; $P < .0001$).

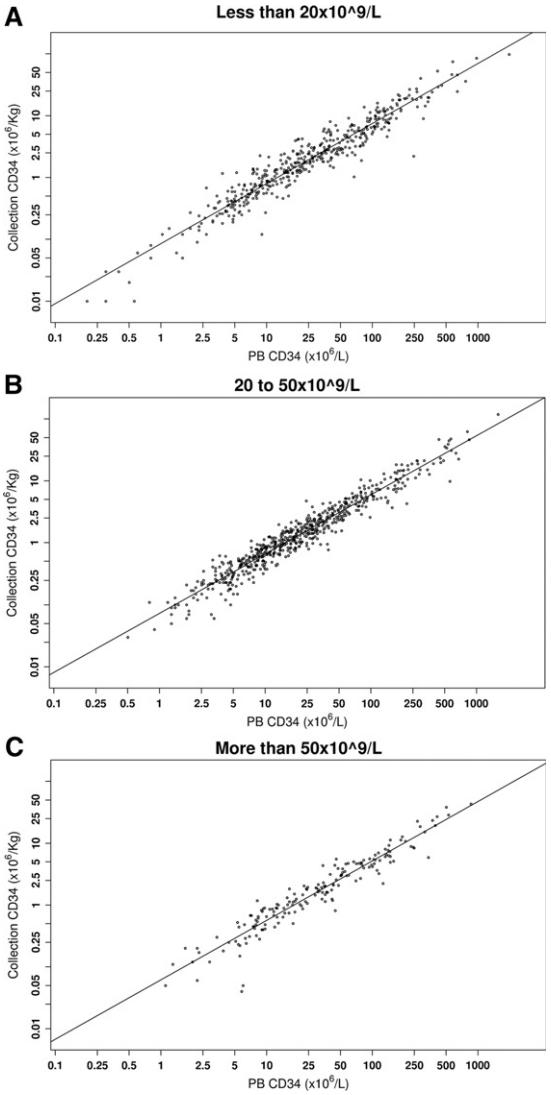

Figure 5. (A) Relationship between collection $CD34^+$ cell count and PB $CD34^+$ cell count for all samples from patients with WCC $<20 \times 10^9$/L (after logarithmic transformation; $y = -2.4500 + 0.9681x$; $R^2 = 92.1\%$; $P < .0001$). (B) Relationship between collection $CD34^+$ cell count and PB $CD34^+$ cell count for all samples from patients with WCC 20-50 $\times$ 10^9/L (after logarithmic transformation; $y = -2.6110 + 0.9538x$; $R^2 = 93.7\%$; $P < .0001$). (C) Relationship between collection $CD34^+$ cell count and PB $CD34^+$ cell count for all samples from patients with WCC $>50 \times 10^9$/L (after logarithmic transformation; $y = -2.7725 + 0.9589x$; $R^2 = 90.4\%$; $P < .0001$).

remain significant in the presence of the other variables, and the final multivariate model obtained was

$$\log(CollCD34) = -2.817 + 0.962\log(PB\ CD34) - 0.186 \times \log(WCC) - 0.002\text{Weight} + \varepsilon$$

After correcting for confounders, PB $CD34^+$ cell count remained a significant predictor for collection $CD34^+$ cell count, with the coefficient staying practically the same. The coefficient for the log(PB CD34) could be interpreted as indicating that a doubling in PB $CD34^+$ cell count would lead to $2^{0.962} = 1.948$-fold higher collection CD34 levels (95% confidence interval, 1.93- to 1.97-fold) if weight and WCC remained the same.

However, although WCC and weight were independent significant predictors, the R^2 value only increased from 91.9% to 93.4%, meaning that they help explain only 1.5% more of the variability in PB $CD34^+$ cell count that was not already explained by the collection $CD34^+$ cell count.

Poor CE Episodes

For all data analyzed, collection episodes were sorted according to ascending CE; nine out of 1,225 demonstrated a CE <20% when PB $CD34^+$ cell count was $>10 \times 10^6$/L. Collection data for these episodes

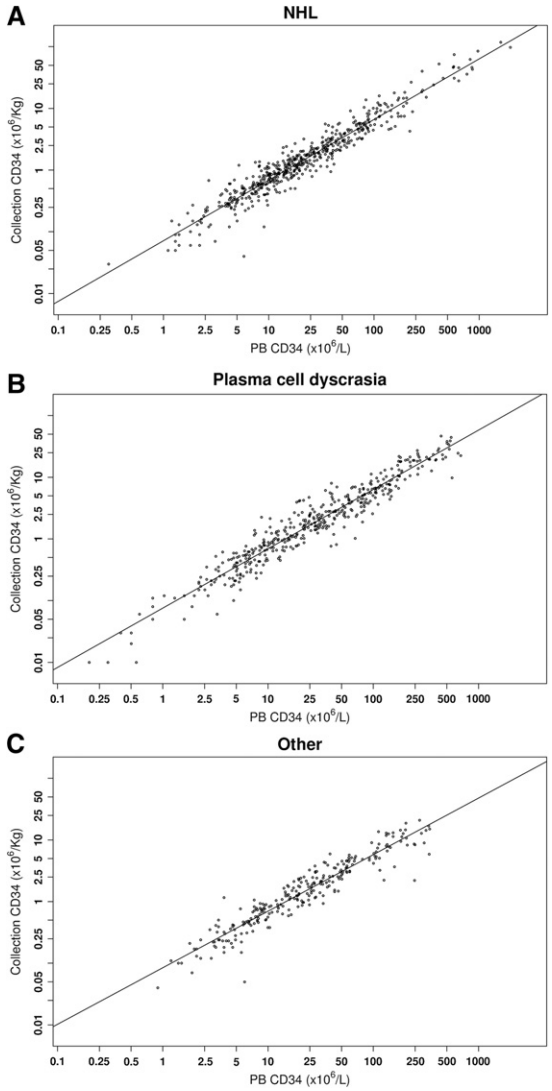

Figure 6. (A) Relationship between collection $CD34^+$ cell count and PB $CD34^+$ cell count for all samples from patients with a diagnosis of NHL (after logarithmic transformation; $y = -2.6365 + 0.9824x$; $R^2 = 92.1\%$; $P < .0001$). (B) Relationship between collection $CD34^+$ cell count and PB $CD34^+$ cell count for all samples from patients with a diagnosis of plasma cell dyscrasia (after logarithmic transformation), $y = -2.5817 + 0.9616x$, $R^2 = 92.3\%$, ($P < .0001$). (C) Relationship between collection $CD34^+$ cells and PB $CD34^+$ cells for all samples from patients with a diagnosis other than plasma cell dyscrasias or NHL (after logarithmic transformation; $y = -2.4711 + 0.9173x$; $R^2 = 90.0\%$; $P < .0001$).

are presented in Table 5. These episodes were categorized according to the variables investigated (ie, WCC, mobilization, weight, and diagnosis). Of these variables, the diagnosis of myelodysplastic syndrome/myeloproliferative disorder (MDS/MPD), as part of the "other" diagnosis category, appeared to have a disproportionately high incidence within this poor CE group. To explore this further, we examined the entire cohort of collection from patients with MDS/MPD (n = 45). In our dataset, this MDS/MPD group comprised patients with a diagnosis of MDS (n = 4), chronic myelogenous leukemia (n = 33), or myelofibrosis (n = 8). The comparative data are displayed in boxplots in Figure 7. The median CE for this MDS/MPD group was significantly reduced compared with the NHL group ($P = .015$) and MM group ($P = .019$). Notably, none of the nine poor CE collections demonstrated an elevated serum albumin or hematocrit level, factors previously reported to have a negative effect on CE [25].

DISCUSSION

In an attempt to identify factors affecting the ability of the PB $CD34^+$ cell count to predict collection $CD34^+$ cell yield, we investigated the impact of mobilization regimen, patient body weight, PB WCC, and diagnosis. We undertook a comprehensive analysis of consecutive collections over a 5-year period.

Our results confirm previous studies that showed the very good predictive power of PB $CD34^+$ cell count for collection $CD34^+$ cell yield. Overall, a significant linear relationship was shown between collection $CD34^+$ cell yield and PB $CD34^+$ cell count. For all data analyzed, our median PB $CD34^+$ cell count of 21.2×10^6/L correlated strongly ($R^2 = 91.9\%$) with our median collection $CD34^+$ yield of 1.48×10^6/kg. Our results show that for all data analyzed, a doubling in PB $CD34^+$ cell count would lead to a $2^{0.9593} = 1.94$-fold higher collection $CD34^+$ cell count. Based on this correlation, Table 6 shows the predicted $CD34^+$ cell yield for a given PB $CD34^+$ cell count. A rule of thumb approximation is PB $CD34^+$ cells $\times$ 0.07 = $CD34^+$ cells. Although a multivariate analysis identified WCC and weight as possible confounders in this univariate model that might affect this approximation, they improved the correlation by only 1.5% on stepwise multivariate analysis (change in R^2 from 91.9% to 93.4%).

When analyzing the effect of mobilization regimen, excellent correlation was shown between PB $CD34^+$ cell count and collection $CD34^+$ cell count regardless of whether patients were mobilized with filgrastim + chemotherapy or filgrastim alone

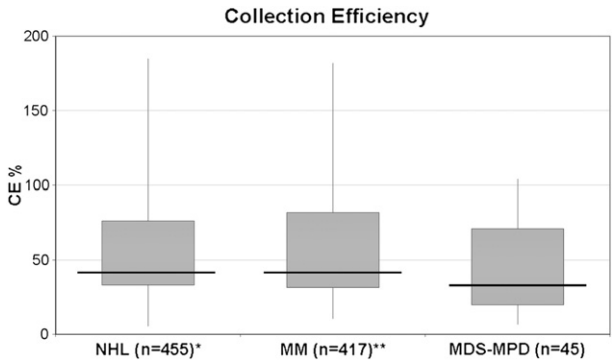

Figure 7. Boxplots showing median, 10th and 90th percentiles, maximum, and minimum CE for collections in patients with NHL, MM, and MDS/MPD. NHL versus MM: P = not significant; *NHL versus MDS-MPD: $P = .015$; **MM versus MDS-MPD: $P = .019$.

Table 6. Predicted CD34+ Cell Yields for Given PB CD34+ Cell Counts

PB CD34+ Cells, $\times 10^6$/L	CD34+ Cell Yield, $\times 10^6$/kg
5	0.36
10	0.70
25	1.68
50	3.26
100	6.34
250	15.26
500	29.68

(R^2 = 91.6% and 89.6%, respectively). Although the purpose of this study was not to compare the effectiveness of mobilization strategies for CD34+ cells, our findings agree with previously reported results demonstrating the greater effectiveness of chemotherapy-based regimens [30]. Clearly, factors that influence physicians' choice of mobilization regimen would have affected the observed differences.

When analyzing the effect of patient body weight, the predictive ability of PB CD34+ cell count for collection CD34+ cell yield was sustained across the different patient body weight ranges, including extremes of weight. A detailed analysis indicates that although subtle differences in the median PB CD34+ cell and collection CD34+ cell counts were seen among the three weight categories, the correlation did not differ significantly (R^2 = >90% for all three weight classes). This is not unexpected given the incorporation of patient body weight into calculated product CD34+ cell yield. At our center, actual body weight is used to calculate the product CD34+ cell content. Incorporation of ideal body weight merits further investigation, given the reported impact of ideal body weight and cell dose on hematopoietic recovery in some published studies [31,32].

Analysis of PB WCC indicates that the small difference in median PB CD34+ cell count and collection CD34+ cell yield among the three WCC categories did not affect the predictive ability of PB CD34+ cell count (R^2 >90% for all three WCC categories). This observation is reassuring, given that extreme WCC values (low or high) is a factor in apheresis machine collection settings.

Analysis of the effect of diagnosis showed higher median PB CD34+ cell counts and collection CD34+ cell yields in patients with plasma cell dyscrasias (MM) compared with NHL and other diagnoses; however, diagnosis did not affect the predictive ability of PB CD34+ cell count in these three diagnostic cohorts (R^2 ≥90% for the three diagnoses). Analysis of MDS/MPD within the "other" diagnostic group is discussed further in the information to follow.

A previous study at our center described poor and excellent CD34 CE phenomenon as false-negative and false-positive outcomes [22]. A false-negative result was described as a good collection when PB CD34+ cell count predicted a poor collection CD34+ cell yield, and a false-positive result was described as a poor collection when PB CD34 predicted a good collection CD34+ cell yield. It is this false-positive group of episodes that causes the most trouble for transplantation teams. Often, an unexpected poor collection results in a subsequent costly collection episode that might not ordinarily occur. Occasionally, it might result in inadequate total CD34+ cell dose across a mobilization regimen, resulting in a second mobilization episode. Although overall our data support that PB CD34+ cell count is highly predictive of collection CD34+ cell yield, in our false-positive group (ie, unexpected poor CE [<20%]), we did identify one group of patients (MDS/MPD) with a significantly lower CE value compared with all other groups, indicating that PB CD34+ cell count did not consistently accurately predict collection CD34+ cell yield in this patient population. This finding is not readily explained. Although the PB CD34+ cell count is often high in this group, why is the CE lower? This phenomenon is not seen in patients with MM, who also often have a high PB CD34+ cell count. Possible technical influences may include unclear mononuclear cells and red cell interface layer, resulting in lower CE values in these patients. Of note, we excluded high serum albumin and hematocrit levels in this group. We could also speculate that biological differences may be influencing the CE, such as differences in the marrow microenvironment, therapy (eg, imatinib), or heterogeneity of the CD34+ cell population given that this group represents disorders of primitive hematopoietic cells (as opposed to disorders of more mature hematopoietic cells, such as NHL and MM). Of note, we did not observe any difference in engraftment kinetics in this group. Future studies could focus on this group and more closely examine such variables as circulating CD34+ cell level before mobilization, comparative effects of filgrastim, major differences in hematocrit, and variations in the red cell interface.

Other technical issues that have not been investigated in detail here include apheresis machine setup and monitoring and the timing of PB CD34+ cell analysis. The true PB CD34+ cell count at the time apheresis actually commences may differ from the PB CD34+ cell count reported earlier that day. Occasionally, apheresis is started as long as several hours after PB sampling due to various factors, including apheresis machine availability and central venous access placement. A delay in starting apheresis should be considered in cases of unexpected poor CD34+ cell yield. That information was not available in this study. Problems associated with improper HPC-A processing after apheresis collection should be considered as well, including inadequate or excessive centrifugation during the volume-reduction step, calculation errors during processing, poorly maintained equipment (eg, scales, centrifuges), and incorrectly programmed

autoprocessing equipment. Well-designed and maintained quality systems should prevent or at least minimize the likelihood of these technical errors. Given that all of our processing was in a cGMP environment, we believe that such issues were of little significance.

We conclude that PB $CD34^+$ cell analysis for prediction of HPC-A $CD34^+$ cell yield is a powerful tool and that such factors as mobilization regimen, PB WCC, and body weight do not influence its reliability. Diagnosis may contribute to variations in CE, any further analysis of this potential phenomenon is warranted. Ongoing monitoring of $CD34^+$ cell yield is vital, and unexpected individual $CD34^+$ CE outliers warrant further investigation. Quality assurance with regular reporting of CE data allows for real-time trend analysis with an enhanced ability to detect sources of CE variability and equipment and/or process malfunction.

ACKNOWLEDGMENTS

Financial disclosure: The authors have nothing to disclose.

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